Induction of class II major histocompatibility complex expression in human multiple myeloma cells by retinoid

Takaomi Sanda, Shinsuke Iida, Satoshi Kayukawa, Ryuzo Ueda

From the Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

This work was supported in part by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare of Japan.

We thank Drs. H. Kagechika (Tokyo Medical and Dental University, Tokyo, Japan) and K. Shudo (Research Foundation ITSUU Laboratory, Tokyo, Japan) for the kind gift of Am80.

Manuscript received May 18, 2006. Accepted August 3, 2006.

Correspondence: Takaomi Sanda, MD, PhD, Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8601. E-mail: tsanda@med.nagoya-cu.ac.jp

ABSTRACT

Class II major histocompatibility complex (MHC II) is normally silenced in plasma/multiple myeloma (MM) cells at the transcriptional level through downregulation of class II transactivator (CIITA), allowing MM cells to escape from immunological responses. Here we demonstrate that a retinoic acid receptor-α/β-selective retinoid Am80 (tamibarotene) could induce the expression of functional MHC II molecules in human MM cell lines. Am80 upregulated expression of the interferon regulatory factor-1 gene, followed by enhancement of CIITA expression. This is the first report demonstrating that retinoid can induce the expression of MHC II in terminally-differentiated plasma/MM cells.

Key words: multiple myeloma, retinoid, MHC II, *CIITA*

Haematologica 2007; 92:115-120

Multiple myeloma (MM) is an intractable B-cell malignancy characterized by clonal proliferation of terminally-differentiated plasma cells in bone marrow.[1,2] MM develops as a result of multistep tumorigenesis caused by chromosomal and genetic alterations.[2] In addition to the low effectiveness of chemotherapy in MM, immunologic responses against MM cannot be detected because most MM cells do not express class II major histocompatibility complex (MHC II), allowing them to escape from direct recognition by idiotype-specific CD4-positive T cells.[3,4] During normal B-cell differentiation to plasma cells, *MHC II* expression is silenced at the transcriptional level through downregulation of a transcriptional regulator, class II transactivator (*CIITA*).[3,5] Transcription of *CIITA* is regulated by four distinct promoters, and promoter III has a binding site for a transcriptional repressor, positive regulatory domain I-binding factor 1/B-lymphocyte-induced maturation protein-1 (PRDI-BF1/Blimp-1), the expression of which is induced during terminal B-cell differentiation into plasma cells.[6] Thus, stage-specific gene expression of *PRDI-BF1* leads to silencing of MHC II expression in plasma cells. Promoter IV has binding sites for interferon-inducible factors such as interferon-regulatory factor-1 (IRF-1) and signal transducing activators of transcription 1 (STAT1), and it regulates interferon-associated expression of CIITA.[7,8]

Retinoids such as all trans-retinoic acid (ATRA) have been reported to inhibit the cell growth of a range of malignancies.[9,10] We have previously demonstrated that a synthetic retinoid Am80 (tamibarotene) could inhibit MM cell growth through upregulation of p21 protein and downregulation of interleukin (IL)-6/ IL-6 receptor.[10] Am80 also inhibited angiogenesis *in vitro* and *in vivo*.[10] Am80 is a retinoic acid receptor (RAR)-α/β-selective retinoid, which does not activate RAR-γ or retinoid X receptors (RXR), thus avoiding unfavorable adverse effects.[11] In addition, Am80 has low affinity for cellular retinoic acid-binding protein (CRABP) and is active against CRABP-rich ATRA-resistant cells. In fact, Tobita *et al.* have reported that 14 of 24 patients with acute promyelocytic leukemia who relapsed after ATRA treatment attained complete remission following Am80 treatment and experienced fewer adverse effects.[12] Thus, Am80 has additional therapeutic advantages compared to those of ATRA. In the present study, we performed gene expression profile analysis to examine the effects of Am80 on a human MM cell line.

Table 1. Upregulated/downregulated genes after treating U266 cells with Am80.

Accession No.	*Gene Symbol*	*Gene name*	*Treated/Control Ratio**
Cell proliferation/cell death			
NM_078467.1	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	2.86
NM_005428.2	VAV1	vav 1 oncogene	2.12
NM_014330.2	PPP1R15A	protein phosphatase 1, regulatory (inhibitor) subunit 15A	2.12
NM_002777.2	PRTN3	proteinase 3	2.02
NM_000308.1	PPGB	protective protein for β-galactosidase	2.01
NM_002961.2	S100A4	S100 calcium binding protein A4	0.47
NM_006113.3	VAV3	vav 3 oncogene	0.47
Signal transduction/cell communication			
NM_016150.3	ASB2	ankyrin repeat and SOCS box-containing 2	6.00
NM_001783.1	CD79A	CD79A antigen (immunoglobulin-associated α)	5.95
NM_017878.1	HRASLS2	HRAS-like suppressor 2	4.96
NM_019111.2	HLA-DRA	major histocompatibility complex, class II, DR α	4.05
NM_001570.2	IRAK2	interleukin-1 receptor-associated kinase 2	3.24
NM_002561.2	P2RX5	purinergic receptor P2X, ligand-gated ion channel, 5	3.17
NM_000889.1	ITGB7	integrin, β 7	3.10
NM_005928.1	MFGE8	milk fat globule-EGF factor 8 protein	3.04
NM_002391.2	MDK	midkine (neurite growth-promoting factor 2)	2.91
NM_002118.3	HLA-DMB	major histocompatibility complex, class II, DM β	2.80
XM_051261.6	PLXNA1	plexin A1	2.41
NM_004355.1	CD74	CD74 antigen (invariant polypeptide of MHC, class II antigen-associated)	2.33
NM_004759.2	MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	2.28
NM_017701.1	ARHGAP8	Rho GTPase activating protein 8	2.27
NM_020979.1	APS	adaptor protein with pleckstrin homology and src homology 2 domains	2.26
NM_002123.2	HLA-DQB1	major histocompatibility complex, class II, DQ β 1	2.22
NM_002664.1	PLEK	pleckstrin	2.21
NM_007181.1	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	2.14
NM_002204.1	ITGA3	integrin, α 3	2.14
XM_291268.2	GRINA	glutamate receptor, ionotropic, N-methyl D-asparate-associated protein 1	2.13
NM_003130.1	SRI	sorcin	2.09
NM_006120.2	HLA-DMA	major histocompatibility complex, class II, DM α	2.07
NM_002730.1	PRKACA	protein kinase, cAMP-dependent, catalytic, α	2.07
NM_033554.2	HLA-DPA1	major histocompatibility complex, class II, DP α 1	2.06
NM_001192.2	TNFRSF17	tumor necrosis factor receptor superfamily, member 17	2.04
NM_002125.3	HLA-DRB5	major histocompatibility complex, class II, DR β 5	2.04
NM_000610.2	CD44	CD44 antigen	0.47
NM_007207.3	DUSP10	dual specificity phosphatase 10	0.46
NM_021205.3	ARHU	ras homolog gene family, member U	0.46
NM_000600.1	IL6	interleukin 6	0.43
NM_177532.1	RASSF6	Ras association (RalGDS/AF-6) domain family 6	0.41
NM_004619.2	TRAF5	TNF receptor-associated factor 5	0.41
NM_002838.2	PTPRC	protein tyrosine phosphatase, receptor type, C	0.37
NM_002351.1	SH2D1A	SH2 domain protein 1A, Duncan's disease	0.32
Transcriptional control			
NM_006509.1	RELB	v-rel reticuloendotheliosis viral oncogene homolog B	4.18
NM_007162.1	TFEB	transcription factor EB	2.63
NM_020062.1	SLC2A4RG	SLC2A4 regulator	2.57
NM_020347.1	LZTFL1	leucine zipper transcription factor-like 1	2.30
NM_006312.1	NCOR2	nuclear receptor co-repressor 2	2.23
NM_005080.2	XBP1	X-box binding protein 1	2.04

**The results are indicated as the ratio of expression by the Am80-treated sample to expression by the untreated control.*

Design and Methods

MHC II-negative human MM cell lines, U266, ILKM-2, ILKM-3, ILKM-8, NOP1, XG7, FR4, SK-MM1, SACHI, NCUMM-2 and NCUMM-3, a B-cell lymphoma cell line, Daudi, and a CD4-positive T-cell leukemia cell line, Jurkat, were used in this study.[10,13,14] Retinoids were prepared as described previously.[10] A cDNA microarray analysis was performed using IntelliGene HS Human expression chips containing 16,600 probes (Takara).[15] Briefly, U266 cells were or were not treated 1 μM Am80 for 24 h, conditions in which Am80 shows approximately 5-10% growth inhibition on U266 cells as previously reported by us.[10] After incubation, total RNA was purified and subjected to a microarray analysis. The results are presented as the ratio of gene expression in the Am80-treated sample to that in the untreated control (T/C ratio). The genes with a T/C ratio of ≥2.00 or ≤0.50 were selected. To validate the results of gene expression analysis, we performed quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using Taqman Gene Expression Assays and the AB7300

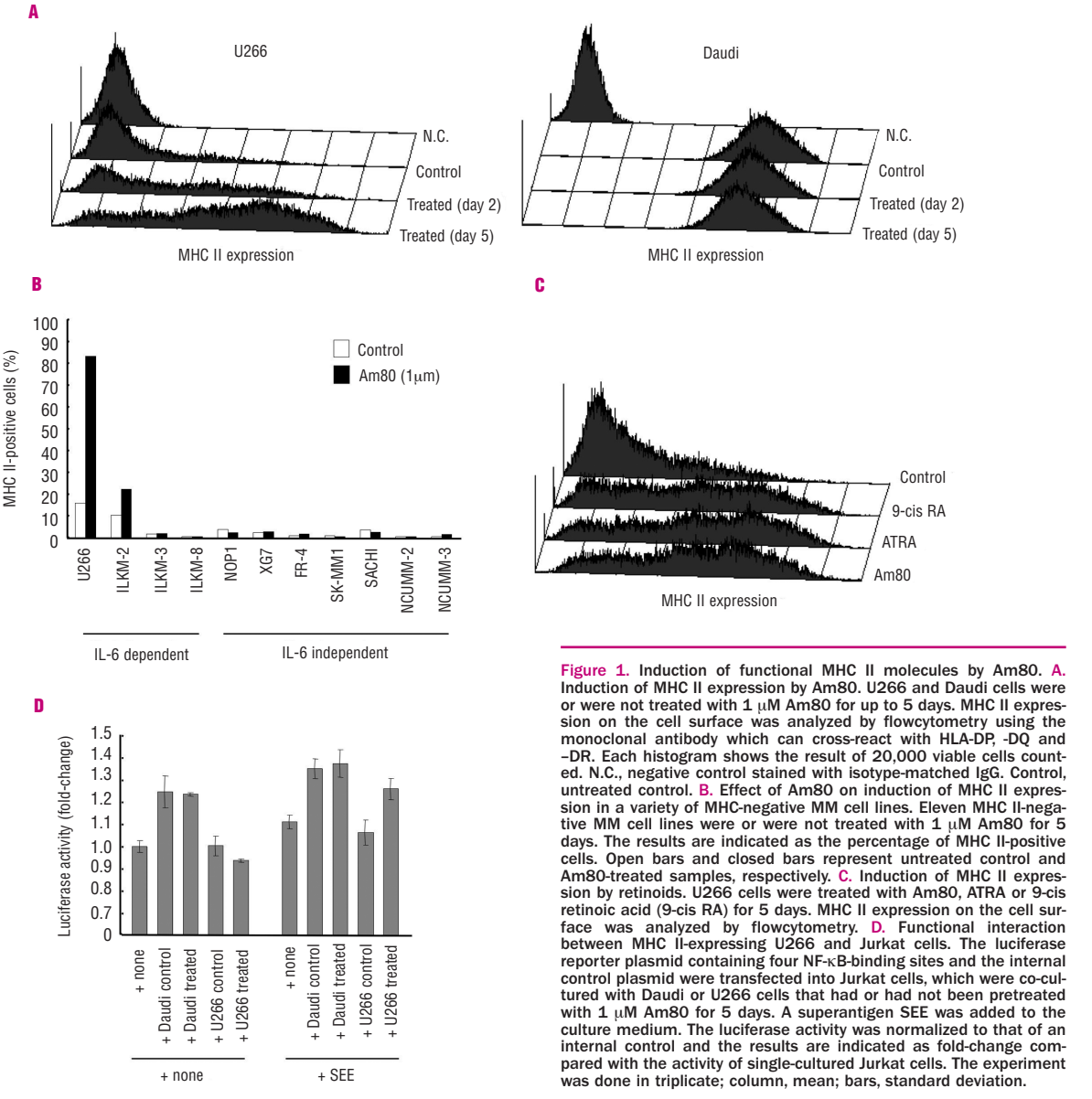

Figure 1. Induction of functional MHC II molecules by Am80. **A.** Induction of MHC II expression by Am80. U266 and Daudi cells were or were not treated with 1 μM Am80 for up to 5 days. MHC II expression on the cell surface was analyzed by flowcytometry using the monoclonal antibody which can cross-react with HLA-DP, -DQ and –DR. Each histogram shows the result of 20,000 viable cells counted. N.C., negative control stained with isotype-matched IgG. Control, untreated control. **B.** Effect of Am80 on induction of MHC II expression in a variety of MHC-negative MM cell lines. Eleven MHC II-negative MM cell lines were or were not treated with 1 μM Am80 for 5 days. The results are indicated as the percentage of MHC II-positive cells. Open bars and closed bars represent untreated control and Am80-treated samples, respectively. **C.** Induction of MHC II expression by retinoids. U266 cells were treated with Am80, ATRA or 9-cis retinoic acid (9-cis RA) for 5 days. MHC II expression on the cell surface was analyzed by flowcytometry. **D.** Functional interaction between MHC II-expressing U266 and Jurkat cells. The luciferase reporter plasmid containing four NF-κB-binding sites and the internal control plasmid were transfected into Jurkat cells, which were co-cultured with Daudi or U266 cells that had or had not been pretreated with 1 μM Am80 for 5 days. A superantigen SEE was added to the culture medium. The luciferase activity was normalized to that of an internal control and the results are indicated as fold-change compared with the activity of single-cultured Jurkat cells. The experiment was done in triplicate; column, mean; bars, standard deviation.

System (Applied Biosystems). The expression value of each gene was normalized to that of β-actin.

To analyze *MHC II* expression, the cells were incubated with staining buffer containing the fluorescein isothiocyanate-conjugated mouse monoclonal antibody which can cross-react with HLA-DP, -DQ and -DR, or isotype-matched control antibody (BD Biosciences), followed by flow cytometry as described elsewhere.[14] For interaction analysis between T-cell receptor (TCR) and MHC II, the reporter plasmid, 4κB-Luc,[16] containing four nuclear factor-κB (NF-κB) binding sites, and the internal control plasmid, pRL-TK, expressing *Renilla* luciferase, were transfected into Jurkat cells using Nucleofector (Amaxa Biosystems). Twenty-four hours after transfection, Jurkat cells were co-cultured with U266 or Daudi cells, which had or had not been pre-treated with 1 μM Am80 for 5 days. The cells were left unstimulated or stimulated with 300 ng/mL of staphylococcal toxin SEE (Toxin Technology). After 24 h incubation, luciferase activity was measured by a luminometer as described previously.[16] In this system, when TCR signaling occurs, luciferase activity in Jurkat cells is induced through NF-κB activation.[17]

Results and Discussion

In order to examine the effects of Am80 on MM cells, we first performed gene expression profile analysis using a cDNA microarray containing 16,600 human genes. Table 1 shows the representative results of gene expression profiles after treatment of U266 cells with Am80. Am80 upregulated *p21* expression and downregulated *IL-6* expression, supporting the previous results.[10] In addition,

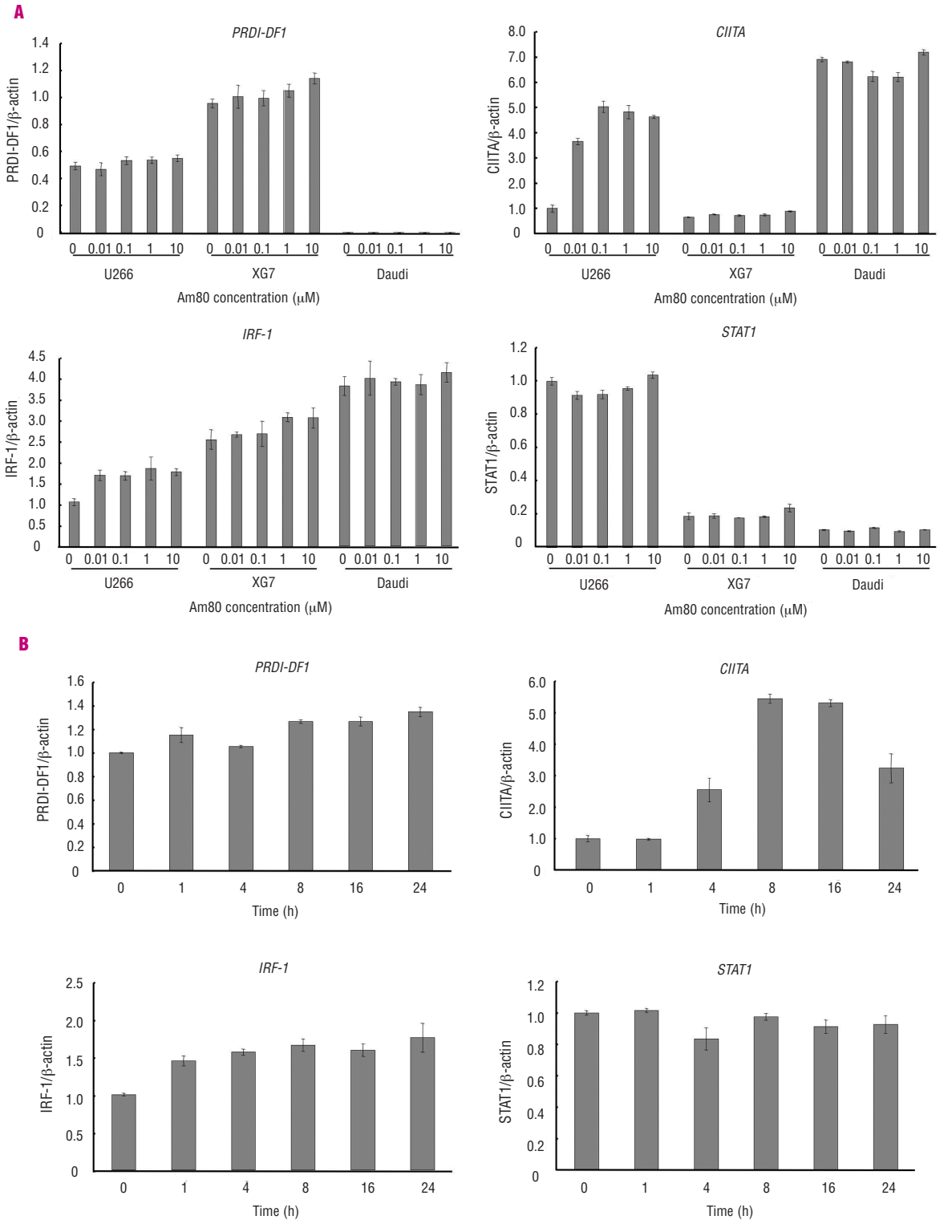

Figure 2. Induction of expression of *IRF-1* and *CIITA* by Am80. **A.** The effect of Am80 on expression of the *IRF-1*, *CIITA*, *PRDI-DF1* and *STAT1* genes. U266, XG7 and Daudi cells were treated with Am80 (0-10 μM) for 24 h. Total RNA was isolated and subjected to quantitative RT-PCR using primers for *IRF-1*, *STAT1*, *PRDI-DF1* or *CIITA*. Expression levels were normalized to the expression level of β-actin and indicated as fold-change compared to the expression of the untreated control. Experiments were done in triplicate; column, mean; bars, standard deviation. **B.** Temporal profile of gene expression after treatment with Am80. U266 cells were treated with 1 μM of Am80 for the indicated times (0-24 h).

Am80 upregulated gene expressions of MHC II molecules including *HLADRA*, *HLADMB*, *CD74*, *HLADQB1*, *HLADMA, HLADPA1* and *HLADRB5*, which prompted us to examine the effect of Am80 on the cell surface expression of MHC II. We used 11 MHC II-negative MM cell lines and a control MHC II-positive B-cell line, Daudi. As shown in Figure 1A, U266 cells did not express MHC II molecules on their surface. Am80 treatment induced the expression of these molecules in a time-dependent manner. On the other hand, in Daudi cells, constitutive expression of MHC II was detected but not modified by Am80 treatment. Among the MM cell lines examined, distinct induction of *MHC II* was observed in U266 and ILKM-2 cells (Figure 1B), both of which are IL-6-dependent cell lines. We then compared the effect of Am80 with that of other retinoids including ATRA and 9-cis retinoic acid (9-cis RA), which can bind to RAR-γ and RXR in addition to RAR-α/β. As shown in Figure 1C, ATRA and 9-cis RA also induced MHC II expression. There was no significant difference among these retinoids, suggesting that RARα/β is important for the induction of *MHC II*.

Next, we examined whether the MHC II molecules induced by Am80 can functionally interact with TCR. We transfected the luciferase reporter plasmid into a CD4-positive T-cell line, Jurkat, and co-cultured them with U266 or Daudi cells, which were or were not pre-treated with Am80. In order to bridge *MHC II* and TCR, the staphylococcal toxin SEE, a superantigen, was added. As shown in Figure 1D, in the presence of Daudi cells, luciferase activity was increased and driven by addition of SEE, whereas U266 cells could not stimulate luciferase activity without pre-treatment with Am80. Importantly, luciferase activity of U266 cells pre-treated with Am80 was increased by the addition of SEE. These results demonstrate that Am80 can induce the expression of functional MHC II molecules in U266 cells.

We then examined the effect of Am80 on *CIITA* gene expression to understand the mode of action of Am80 on MHC II induction. We used U266, Daudi and a MM cell line XG7, in which MHC II expression could not be induced by Am80 (Figure 1B). As shown in Figure 2A, *PRDI-DF1* was highly expressed in U266 and XG7 cells, and *CIITA* expression was suppressed. Although Am80 treatment did not modify the expression of *PRDI-DF1*, Am80 induced a 4-fold increase in *CIITA* expression in U266 cells, but not in XG7 cells. In contrast, *PRDI-DF1* was completely silenced in Daudi cells, and *CIITA* was highly expressed. Am80 treatment did not upregulate *CIITA* expression in Daudi cells. ATRA and 9-cis RA could induce expression of *IRF-1* and *CIITA* in U266 cells (*data not shown*).

We then examined the effect of Am80 on expression of the interferon-inducible genes such as *IRF-1* and *STAT1*. In U266, but not XG7 or Daudi cells, Am80 induced a 1.6-fold increase in IRF-1 expression, whereas Am80 had no effect on STAT1 expression in either cell line (Figure 2A). The temporal profile of gene expression by U266 cells showed upregulation of *IRF-1* at 1 h after the treatment with Am80 and upregulation of *CIITA* from 4 h after treatment (Figure 2B). *IRF-1* has been reported to be induced by retinoid at the transcriptional level through the binding of RAR to the *IRF-1* promoter.[18,19] Although it remains unclear why Am80 could not induce the expression of *IRF-1* in XG7 cells, these findings suggest that Am80 directly enhances the expression of *IRF-1*, which is followed by secondary induction of *CIITA* expression without changing the *PRDI-DF1*.

In conclusion, Am80 has an immunomodulatory effect in addition to anti-proliferative and anti-angiogenic activities. Although Am80 did not induce MHC II expression in all MM cell lines, it might enhance the immunogenicity of MM cells *in vivo* through the induction of MHC II expression. Since Am80 appears to be a safe and practical agent, which has many therapeutic advantages over other retinoids, it could be used as a chemopreventive agent from the earliest stage of MM.

Author Contributions

SI: directed this study and performed several experiments; SK: performed microarray analysis: RU: directed this study.

Conflict of Interest

The authors reported no potential conflicts of interest.

References

1. Sirohi B, Powles R. Multiple myeloma. Lancet 2004;363:875-87.
2. Iida S, Ueda R. Multistep tumorigenesis of multiple myeloma: its molecular delineation. Int J Hematol 2003; 77:207-12.
3. Silacci P, Mottet A, Steimle V, Reith W, Mach B. Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. J Exp Med 1994;180:1329-36.
4. Lauritzsen GF, Weiss S, Dembic Z, Bogen B. Naive idiotype-specific CD4+ T cells and immunosurveillance of B-cell tumors. Proc Natl Acad Sci USA 1994;91:5700-4.
5. Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B. Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. Embo J 1997; 16:2851-60.
6. Ghosh N, Gyory I, Wright G, Wood J, Wright KL. Positive regulatory domain I binding factor 1 silences class II transactivator expression in multiple myeloma cells. J Biol Chem 2001;276:15264-8.
7. Morris AC, Beresford GW, Mooney MR, Boss JM. Kinetics of a gamma interferon response: expression and assembly of CIITA promoter IV and inhibition by methylation. Mol Cell Biol 2002;22:4781-91.
8. Piskurich JF, Wang Y, Linhoff MW, White LC, Ting JP. Identification of distinct regions of 5' flanking DNA that mediate constitutive, IFN-γ, STAT1, and TGF-β-regulated expression of the class II transactivator gene. J Immunol 1998;160:233-40.
9. Freemantle SJ, Spinella MJ, Dmitovsky E. Retinoids in cancer therapy and chemoprevention: promise and

resistance. Oncogene 2003;22:7305-15
10. Sanda T, Kuwano T, Nakao S, Iida S, Ishida T, Komatsu H, et al. Anti-myeloma effects of a novel synthetic retinoid Am80 (Tamibarotene) through inhibition of angiogenesis. Leukemia 2005;19:901-9.
11. Takagi K, Suganuma M, Kagechika H, Shudo K, Ninomiya M, Muto Y, et al. Inhibition of ornithine decarboxylase induction by retinobenzoic acids in relation to their binding affinities to cellular retinoid-binding proteins. J Cancer Res Clin Oncol 1988;114:221-4.
12. Tobita T, Takeshita A, Kitamura K, Ohnishi K, Yanagi M, Hiraoka A, et al. Treatment with a new synthetic retinoid, Am80, of acute promyelocytic leukemia relapsed from complete remission induced by all-trans retinoic acid. Blood 1997;90:967-73.
13. Suzuki, A, Iida, S, Kato-Uranishi, M, Tajima, E, Zhan, F, Hanamura, I, et al. ARK5 is transcriptionally regulated by the Large-MAF family and mediates IGF-1-induced cell invasion in multiple myeloma: ARK5 as a new molecular determinant of malignant multiple myeloma. Oncogene 2005;24:6936-44.
14. Sanda T, Asamitsu K, Ogura H, Iida S, Utsunomiya A, Ueda R, et al. Induction of cell death in adult T-cell leukemia cells by a novel IkB kinase inhibitor. Leukemia 2006;20:590-8.
15. Arai M, Kondoh N, Imazaki N, Hada A, Hatsuse K, Kimura F, et al. Transformation-associated gene regulation by ATF6a during hepatocarcinogenesis. FEBS Lett 2006; 580: 184-90.
16. Sanda T, Iida S, Ogura H, Asamitsu K, Murata T, Bacon, KB, et al. Growth inhibition of multiple myeloma cells by a novel IkB kinase inhibitor. Clin Cancer Res 2005;11: 1974-82.
17. Weil R, Israel A. T-cell-receptor- and B-cell-receptor-mediated activation of NF-kappaB in lymphocytes. Curr Opin Immunol 2004;16:374-81.
18. Pelicano L, Li F, Schindler C, Chelbi-Alix MK. Retinoic acid enhances the expression of interferon-induced proteins: evidence for multiple mechanisms of action. Oncogene 1997; 15:2349-59.
19. Matikainen S, Ronni T, Hurme M, Pine R, Julkunen I. Retinoic acid activates interferon regulatory factor-1 gene expression in myeloid cells. Blood 1996;88:114-23.